INFORMATION REGARDING ANTIMICROBIAL PRODUCT EFFICACY DATA

Warning: The information in this document was valid when written in the 1990's, but the references and information it contains may not reflect current law or practice. Please confirm any information by referring to the other parts of this manual.

"Antimicrobial" means those pesticides used to control, prevent, destroy, or mitigate bacteria, fungi, or viruses in any environment except those on or in living animals (including man), living plants, processed food, beverages, pharmaceuticals, and cosmetics.

Antimicrobial agents include disinfectants, sanitizers, non-liquid sterilants, virucides, bacteriostats, algaecides, fungicides, and fungistats used on inanimate surfaces and in aquatic environments. Also included are pH adjusters and modifiers.

Liquid chemical sterilants (except ethylene oxide) labeled for use on medical instruments introduced directly into the human body or in contact with intact mucuous membranes are not considered pesticides.

GENERAL CONSIDERATIONS

Validating the Test Methods

The efficacy of most antimicrobial agents cannot be predicted on the basis of their chemical composition or formulation. It can be determined only by adequate microbiological testing.

Depending upon the type of antimicrobial agent, target microorganisms, and site to be treated, all tests should be designed to include those factors normally encountered in the use pattern intended for the product; e.g., presence or absence of organic soil or other interfering conditions, temperature, exposure period, and the number of times, or duration that the use solution can be used or reused. The actual test procedure will vary according to the characteristics of the product, the target organism(s) and the pattern of use intended.

A variety of standardized laboratory test methods for evaluating the efficacy of antimicrobial agents has been adopted by AOAC International (formerly the Association of Official Analytical Chemists). It is recommended that these standard methods be

employed whenever applicable for determining the efficacy of antimicrobial pesticides and developing the efficacy data necessary for California registration. In some cases, modification of these standard methods will be necessary to reflect use conditions applicable to the particular product.

If an applicable AOAC method is not available for a particular product type or use, it is recommended that an applicable standard or established method adopted by USEPA be used, e.g. standard methods from the American Society for Testing and Materials (ASTM). It should be noted, however, that the applicant is responsible for the validity of the results and the applicability of the test method selected.

If no established method is available, or if major modification to a standard method is required, the applicant may submit a proposed protocol for review. This protocol is routed into the evaluation process with an EP suffix on the tracking ID#.

Test Substance

Tests involving antimicrobial pesticides should be conducted with the formulation to be offered for sale. The test results must demonstrate the effectiveness of the product's formulation when used according to the proposed label's directions for which registration is sought.

Exposure Period (Contact Time)

Claimed exposure times must be substantiated with data from appropriate tests. If the labeling of a product contains directions for use at exposure periods shorter or longer than those specified in the applicable test method, the test method should be modified to conform to the exposure period described in the label directions. When no exposure period is referenced on the label, it is assumed by the evaluation scientist to be 10 minutes for disinfectants and 30 seconds for sanitizers.

Hard Water Claims

If effectiveness in hard water is claimed on the product label, the product must be tested by the appropriate method in water at the claimed hardness level. Water hardness should be expressed in terms of ppm of calcium carbonate.

Organic Soil

If a product is intended for use as a one-step cleaner-disinfectant, cleaner-sanitizer, or in the presence of organic soils, the product must be tested by the appropriate method in the presence of a representative organic soil normally associated with the site of use, such as five percent blood serum for use in medical environments, chicken manure in a poultry house, or horse manure in a stable.

Soap Scum

If the product claims effectiveness as a disinfectant or sanitizer in the presence of soap scum, the study must be conducted in the presence of representative soap residue, eg. 0.005% sodium stearate.

Reuse of Product

If the product label claims reuse of the use solution for a period of time more than one day, such claims must be substantiated with efficacy data demonstrating how many times a prepared use solution of the product can be used and reused before a fresh solution should be prepared. The data must demonstrate the claimed effectiveness of the use solution that has undergone simulated re-use stressing with cumulative bioburden over the period of time specified in the directions.

If a product label claims use to disinfect barber and beauty salon equipment, re-use data derived from samples that are stressed for seven days, are required even if the label does not recommend re-use. This data requirements is waived if the label instructs users to change the solution at least daily. This requirement is due to the California Board of Barbering and Cosmetology regulation Section 979, allowing disinfecting solutions to be used for up to one week without changing.

Adverse Effects

Data describing the nature and extent of any adverse effects observed on treated materials or surfaces resulting from treatment with the antimicrobial pesticides (e.g., staining, discoloration, corrosion, etc.) must be provided as part of the efficacy report. Laboratory reports on failure of the tests should also be included in the submission.

Reporting of Efficacy Data

Systematic and complete descriptions of the test employed and the results obtained are essential for proper review and evaluation of product performance by DPR. All test reports must include identification of the testing laboratory or organization, when and where the tests were conducted, and the name(s) and signature(s) of the person(s) responsible for conducting the test.

If the methods employed to develop efficacy data are standard methods (such as standard AOAC test), certain minimal information must be provided in the test report. The report must include, but is not limited to, the following:

- Test method employed and any modifications.
- Test microorganisms employed including identification of the specific strain ATCC or other.

- Concentration of the product tested and the preparation procedure.
- Preparation date or manufacturing date of each product batch.
- Testing date.
- Subculture media.
- Exposure period (contact time) and temperature.
- Incubation time and temperature.
- Phenol resistance data is no longer required as outlined in USEPA PR Notice 2001-4.
- Identification of all material of procedural options employed where such choice is permitted or recommended in the test method selected. For example, growth media, drying time for inoculated carriers, neutralizer and/or subculture media, secondary subculturing.
- Complete report of results obtained for each individual replication.
- Any control data essential to establish the validity of the test.

If standard methods are significantly modified to support specific claims and/or patterns for a product, the protocol modifying the test must be provided in specific detail with the test report.

If standard methods, or modification thereto, are not employed to develop efficacy data (such as actual in-use or many kinds of simulated-use testing), complete testing protocols must be submitted with the test reports. All materials and procedures employed in testing should be described. (Note: The format of the report may be modeled after original research reports published in technical or scientific journals).

Where references to published reports or papers are made, copies or reprints of such references should be provided with test reports.

In reporting the results of in-use tests or simulated in-use tests, data on adverse effects, including the nature and extent of such effects, should also be included in the report.

EFFICACY DATA REQUIREMENTS FOR ANTIMICROBIAL PRODUCTS

Basic Requirements

For reference, use U.S. EPA Pesticide Assessment Guidelines, Subdivision G.

Specific efficacy data requirements, testing protocols, test methods and standards for antimicrobial agents are those adopted by U.S. EPA and published in the Pesticide Assessment Guidelines, Subdivision G (Product Performance), Sections 91-1 through 91-97, dated October 1982.

Categories of products for uses not covered by the guidelines shall be supported by appropriate data developed from tests using scientific methods and procedures acceptable

to the Director. When in doubt, the applicant may contact the Department to discuss the type of data that would be required and acceptable test methods applicable to their product. Written protocols of proposed tests for efficacy may be submitted for review before initiating the test.

Following are examples of products or uses not presently covered by U.S. EPA guidelines:

- Antimicrobial agents used in waterbeds to control the growth of slime-forming organisms.
- Antimicrobial agents used in aquariums.
- Adjuvants such as stabilizers used in swimming pools to stabilize swimming pool water disinfectants.
- Antimicrobial agents used in controlling the growth of slime-forming organisms in drip irrigation systems.

Antimicrobial Products Requiring Confirmatory Data Only (Discontinued in 2006)

Confirmatory efficacy data are required for certain antimicrobial products labeled for use only as a disinfectant or sanitizer.

There are two commonly encountered situations in which an applicant is permitted to use previously submitted basic efficacy data another registrant's basic efficacy data and submit only confirmatory data (supplemental to the basic reference data) to support the registration of their antimicrobial product. These two specific situations and the required confirmatory data are described on the following page. Confirmatory data are required on the applicant's own finished product, as offered for sale, to demonstrate their ability to produce an effective product.

When the test methodology utilized in deriving the original supporting efficacy data were modified to include additional elements not specified in the recommended method, such as organic soil, hard water, longer or shorter contact time, etc., the confirmatory data must also be produced under similarly modified conditions. The specified confirmatory data are required to be developed at the dilution and condition which represents the highest level of efficacy and most stringent condition claimed on the label.

- Duplicated or identical product formulations:

The formulation for the product proposed for registration is the same as for one which the cited data were derived from, but which is manufactured or packaged by a different company. In addition, the label claims and directions for use for both products should be basically identical.

- Minor formulation change in registered product:

Confirmatory data are required in most cases with minor formulation changes. However, there are exceptions to the rule. For example, substituting different kinds of dyes and fragrances, in most situations, will not require confirmatory data.

Confirmatory Data Requirement

The following are confirmatory tests and performance standards for the various types of disinfectants and sanitizers:

Disinfectants (limited efficacy)

Confirmatory test standard:

Ten carriers on each of two samples representing two different batches must be tested against either Staphylococcus aureus or Salmonella choleraesuis, depending upon the microorganisms against which the activity of the product is limited. The AOAC Use-Dilution Method for liquids or the AOAC Germicidal Spray Products Test for spray products should be used. (Note: Products with claims against Gram positive bacteria should be tested against S.aureus; products with claims against Gram negative bacteria should be tested against S. cholerasuis.)

Confirmatory performance standard:

Killing on all carriers is required.

Disinfectants (general or broad spectrum efficacy)

Confirmatory test standard:

Ten carriers on each of two samples representing two different batches tested against both Staphylococcus aureus and Salmonella choleraesuis using the AOAC Use-Dilution Method for liquid products, or the AOAC Germicidal Spray Products Test for spray products are required.

Confirmatory performance standard:

Killing on all carriers is required.

Disinfectants (hospital or medical management efficacy)

Confirmatory test standard:

Ten carriers on each of two samples representing two different batches tested against Salmonella choleraesuis, Staphylococcus aureus and Pseudomonas aeruginosa. The AOAC Use-Dilution Method for liquid products, or the AOAC Germicidal Spray Products Test for spray products should be used.

Confirmatory performance standard:

Killing on all 100% carriers is required.

Sanitizer for previously cleaned food-contact surfaces

Confirmatory tests standard:

One test on one sample, with or without hard water (depending on label claims), is required using either the AOAC Germicidal and Detergent Sanitizer Test against Escherichia coli for quaternary ammonium compounds, chlorinated trisodium phosphate and anionic detergent-acid formulations, or the AOAC Available Chlorine Germicidal Equivalent Concentration Test against Salmonella typhi for iodophor, mixed halides, and chlorine-bearing chemicals.

Confirmatory performance standard:

For halide products, test results must show product concentration equivalent in activity to 50, 100, and 200 ppm of available chlorine (the reference standard sodium hypochlorite). For other chemical products, acceptable results must demonstrate a 99.999% reduction in the number of each test microorganism within 30 seconds contact time, or the length of contact time as specified on the label. The results must be reported according to actual count and percentage reduction over the control.

Reference U.S. EPA's Confirmatory Efficacy Data Requirements DIS/TSS-5 dated September 22, 1982.

Antimicrobial Products NOT Requiring Confirmatory Data

Confirmatory efficacy data are not required for antimicrobial products not labeled as a disinfectant or sanitizer for use on food contact surfaces. Confirmatory efficacy data are not required for products which are merely repackaged, relabeled, or simple aqueous dilutions of a product already registered for which there are data on file to support product registration.

Disinfectants and sanitizers with formulations and claims identical to a registered product and manufactured by the same formulator as the registered product, do not require confirmatory data.

Disinfectants and sanitizers that do not require confirmatory data must be accompanied by the following documents:

- A document substantiating that the product was formulated by the same manufacturer who formulated the product which the basic efficacy data were derived. The U.S. EPA establishment number for both products should be the same. (Note: This is not required for repackaged products or products formulated by simple aqueous dilution.)
- Specific references and authorization to cite the data developed for the original product.
- If the product is a pressurized spray, all materials and devices must be shown to be identical to those utilized by the basic registrant.

All documentation submitted must be verified.

Sanitizer and Disinfectant Label Requirements

Reference U.S. EPA Pesticide Assessment Guidelines, Subdivision H, available on microfiche.

Labels for sanitizer and disinfectants often lack certain basic elements in the directions for use. Following are some of these elements which are most often neglected, along with the recommended action:

- Sanitizer for food contact surfaces:

Potable water rinse after sanitizing food contact surfaces is no longer allowed on the label. Refer to the USEPA Label Improvement notice for sanitizers issued July 25, 1986, to registrants of antimicrobial products used as food contact surface sanitizer. This Notice was issued in accordance with the Label improvement Program, PR Notice 80-1. For other label requirements, refer to DIS/TSS-11.

- Disinfectants for use on environmental surfaces:

Use directions should indicate a minimum contact time with the disinfectant and should clearly indicate the method of application, e.g. wiping, mopping, or spraying. For reference, see DIS/TSS-15.

- Disinfectants and sanitizer for non-food contact surfaces:

If wiping off or rinsing after treatment is to be recommended, a minimum contact time with the disinfectant or the sanitizer should be specified on the label.

- Disinfectants for use on farm premises:

Use directions should require removal of all animals from premises, litter and manure from floors, and emptying of all feeding and watering troughs prior to treatment. For label reference, see DIS/TSS-18.

- Air sanitizer:

If removal of people or animals before releasing the air sanitizer in a closed area is required or recommended, a minimal reentry period should be specified on the label and such a reentry period should be supported by adequate data. For other label requirements, refer to DIS/TSS-11.

- Virucidal claims must be qualified by designating each specific virus against which the product has been tested and shown to be effective and to indicate that the activity occurs only on environmental surfaces.

pH Control Buffers

Data must show how much product is required to buffer 100 gallons of water, having hardness equivalent to 175 ppm as calcium carbonate, to the pH recommended on the label, except for swimming pool products. If no pH is recommended on the label, then pH may be used as the criteria, except for swimming pools. Buffering capacity for other pHs may be requested in some cases. pH control buffers are registered as spray adjuvants in California.

Aquatic Use Antimicrobial Products

Antimicrobial products for use in aquatic conditions include disinfectants, bactericides, fungicides, and algaecides for use in various aquatic sites such as swimming pools, spas, fountains, decorative ponds, reservoirs, waterways, water cooling systems, air washers, brewery pasteurizers, retort systems, pulp and paper mill water systems, secondary oil recovery systems, waterbeds, drip irrigation systems, etc.

Except for a few uses, such as pulp and paper mill water systems and secondary oil recovery systems, the data requirements for these types of products are as follows:

1. Presumptive laboratory data derived from the product tested against representative problem-causing organisms normally found in the target water systems and any other

additional organisms claimed on the proposed label. These data should demonstrate the minimum biocidal and/or biostatic concentrations of the product against the tested organisms under a defined contact time. The AOAC Use Dilution Test method and other methods used to determine the minimum inhibitory concentrations are acceptable for determining the presumptive data.

2. In-use or simulated in-use data derived from a minimum of two different units of each type of water system claimed on the proposed label. In-use studies should be conducted at test rates, frequencies of treatment, and other specified use conditions according to the directions for use and for a defined period of time appropriate for the specific use sites.

In general, disinfectants for use in swimming pools need to be tested for the duration of the entire swimming season; algaecides for use to winterize swimming pools need to be tested for the duration of the entire off season, i.e. from late autumn to early spring; and algaecides for use in swimming pools and most water cooling systems need to be tested for a minimum period of 30 days in systems with existing algae problems.

The duration of the studies may also be lengthened or shortened to conform with specific label directions on the duration of the effective period per dose regimen. For example, if a biocide used in treating the condensation drip pans in refrigeration units claim effectiveness for at least 3 months, each unit/dose must be tested for a period of 3 months. Depending on the protocol used, untreated control units may be required in order to demonstrate by comparison the effectiveness of the treated units.

For pulp and paper mill water systems, if the ASTM Standard Test Method for Efficacy of Slimicides for the Paper Industry - Bacterial and Fungal Slime (E 599 and E600, or E1839-96) is used, no in-use studies are required unless the laboratory studies are inconclusive.

For secondary oil recovery water systems, no in-use studies are required. The bacteriostatic and time-kill tests in the American Petroleum Institute (API) Recommended Practice for Biological Analyses of Subsurface Injection Water Tests are acceptable methods and should be carried out in duplicate.

Antimicrobial Preservatives or Industrial Processing

Antimicrobial products are also used as preservatives, especially during industrial processing. Some examples of the materials to be preserved are paints and stains (in-can preservatives), paint films, metal working fluids, fuel, drilling muds, adhesives, latex emulsions, caulks and sealants, polishes, cleansers, cosmetics, pesticides, sponges.

The data requirements for this type of product generally include in-use or simulated in-use data tested on representative samples of the materials to be preserved at the lowest label recommended use rate for the longest period of time claimed on the label. Untreated control samples are required. The inocula used may either be spoiled materials or isolates

from spoiled materials. Tests against bacteria and fungi must be conducted separately for the data to be acceptable.

Presumptive laboratory data are also required for certain uses such as in fuel additives and additives for sugar mills. For most other uses, presumptive laboratory data are not strictly necessary, but may be used as the basis for establishing antimicrobial activities of the products as grounds for requesting conditional registration.

For additional reference and specific tests for specific uses, see the U.S. EPA Pesticide Assessment Guidelines, Subdivision G.

ANTIMICROBIALS, REQUIREMENT FOR DATA FROM IN-USE TESTS CONDUCTED UNDER CALIFORNIA CONDITIONS

Testing conducted under California conditions is not usually a concern for antimicrobial products. Products, such as many of the antimicrobials, intended for indoor use are not affected by climatic or environmental conditions. Furthermore, because California is a large state with a wide range of climatic and environmental conditions, data generated in locations that are not in extreme climate conditions, will in general be bridgeable to California conditions.

DPR would request repetition of in-use studies, if the submitted studies were generated in extreme environmental conditions not normally encountered in California.

EFFICACY DATA REQUIREMENTS - PRODUCTS FOR USE ON HARD SURFACES

Level of Activity	Test Method, AOAC	Test Microorganisms	Total No.of Carriers	Performance Standard
STERILIZERS	Sporicide Test Method	Bacillus subtilis (ATCC 19659) **and** Clostridiam sporogenes (ATCC 3584)	720 (a)	Killing on all 720 carriers
DISINFECTANTS - Limited Efficacy	Use-Dilution (solids & liquids) **or** Germicidal Spray (sprays)	Salmonella choleraesuis (ATCC 1078) for gram-negative **or** Staphyloccus aureus (ATCC 6538) for gram-positive	180 (b)	Must kill 59 out of each 60 carriers

- General or Broad Spectrum Efficacy	Use-Dilution (solids & liquids) **or** Germicidal Spray (sprays)	Salmonella choleraesuis (ATCC 1078) **and** Staphylococcus aureus (ATCC 6538)	360 (c)	Must kill 59 out of each 60 carriers
- Hospital or Medical Environment Efficacy	Use-Dilution (solids & liquids) **or** Germicidal Spray (sprays)	Salmonella choleraesuis (ATCC 1078) **and** Staohylococcus aureus (ATCC 6538) **and** Pseudomonas aeruginosa (ATCC 15442)	540 (d)	Must kill 59 out of each 60 carriers
SANITIZERS - For Nonfood Contact Surfaces	USEPA's Test Method (91-30,D8)	Staphylococcus aureus (ATCC 6538) **and** Klebsiella pneumoniae (ATCC 4352) (e)	one test on each representative surface	99.9% reduction compared to control within 5 minutes
- For Food Contact Surfaces				
* For Halides	Available Chlorine Germicidal Equivalent Concentration Method	Salmonella typhi (ATCC 6539)	one test on each of 3 samples	concentration equivalent to 50, 100 and 200 ppm available chlorine
* For Other Products	Germicidal and Detergent Sanitizers Method	Escherichia coli (ATCC 11229) **and** Staphylococcus aureus (ATCC 6538)	one test on each of 3 samples	99.999% reduction in each test organism within 30 seconds

Reference: USEPA Pesticide Assessment Guidelines Subdivision G: Product Performance, Part 91-2. (a) 60 carriers x 2 types of surfaces x 3 samples x 2 types of test organisms = 720 carriers.
(b) 60 carriers x 1 type of surface x 3 samples x 1 type of test organism = 180 carriers.
(c) 60 carriers x 1 type of surface x 3 samples x 2 types of test organism = 360 carriers.
(d) 60 carriers x 1 type of surface x 3 samples x 3 types of test organism = 540 carriers.
(e) Enterobacter aerogenes (ATCC 13048) may be substituted for Klebsiella pneumoniae.